REMARKS

This is in response to the official action dated August 26, 2011. Reconsideration in view of the following remarks is respectfully requested.

Claim Status

Claims 9 and 24-36 stand rejected. Claim 37 is new. Claims 12-18 and 36 stand withdrawn. Claims 1-8, 10, 11, and 19–23 are canceled.

Claim Rejection – 35 USC § 102(b) – Fogolin et al/Claim 35

Examiner asserts that **Fogolin**'s disclosure of a GM-CSF glycoprotein in **non-human** cells such as COS (monkey) and CHO (hamster) anticipates the optimally glycosylated glycoprotein highly active in human cells as claimed in claim 35.

Applicant respectfully disagrees. Significantly, the proteins differ substantially in their carbohydrate structures. The GM-CSF of claim 35 has a **human** glycosylation pattern (in contrast to GM-CSF produced by Fogolin in **non-human** CHO or COS cells). More significantly, the GM-CSF of claim 35 differs in that the glycosylation is structurally optimized in its sialic acid content to provide higher activity, as may be achieved e.g. by expression in NM-F9 cells in a medium supplemented with 90 mM ManNAc, according to the present disclosure.

Notably, unlike the cells used by **Fogolin et al.,** NM-F9 is a **human**

myeloid leukemia cell line which provides the GM-CSF produced therein with a **human** glycosylation pattern. Instead, **Fogolin et al.** uses the monkey cell line COS and the hamster cell line CHO, which produce a monkey or hamster glycosylation pattern, respectively.

Since the glycosylation activities are different for different species, a glycoprotein obtained from a cell line of one species differs in the carbohydrate structures (glycosylation pattern) from the same glycoprotein produced by a cell line of another species. Differences in glycosylation affect the properties of the glycoprotein, for example, carbohydrate structures uncommon in human glycoproteins can induce adverse immune reactions if administered to a patient.

Specifically, glycoproteins produced by CHO cells (hamster) frequently have NeuGc and Gal-Gal structures which are not present in the human glycosylation and thus are prone to cause immune reactions against the glycoproteins. NeuGc which is completely missing in human glycosylation is also present in glycoproteins produced by COS cells (monkey). Furthermore, the CHO cell line has a low sialylation activity and is not capable of producing α2,6-coupled sialic acid residues, unlike the majority of the sialic acid residues provided by the NM-F9 cell line used in the claimed process to produce the claimed product, which are α2,6-coupled.

Due to the use of an expression cell line having a defect in the sialic acid biosynthesis (the NM-F9 cells) and a specific concentration of the sialic acid precursor

additive in the culture medium (90 mM ManNAc), GM-CSF with a human glycosylation pattern and a very specific amount of sialic acid residues in its carbohydrate structure can be formed. As demonstrated in the example of the present application, GM-CSF having this specific degree of sialylation has the highest activity for inducing proliferation of TF1 cells.

GM-CSF with a different degree of sialylation results in a lower activity. As demonstrated in the examples, this is true both for GM-CSF having a lower degree of sialylation (lower concentration of ManNAc used during cell culture), as well as GM-CSF having a **higher** degree of sialylation (90 mM ManNAc **plus fetuin** as sialic acid donor): Example 6, Figure 11 shows the amount of non-sialylated GM-CSF in relation to the ManNAc (A) and fetuin (B) concentration used, and Figures 12 and 13 show the relative cell proliferation in relation to the sialylation form of GM-CSF used.

Accordingly, the glycoprotein producible by a process involving human cells and a specific selection process as claimed (human cell line NM-F9 with 90 mM ManNAc) to provide a specific sialic acid content in the novel highly active product clearly differs from **Fogolin**'s GM-CSF.

Neither F**ogolin** nor any other prior art document discloses the claimed structurally different specificly sialylated form of GM-CSF which is characterized by its unexpectedly high activity to stimulate proliferation.

Claim Rejection – 35 USC § 102(f) – Sigma Aldrich Catalog/Claim 35

As discussed above for the rejection under 102(b) in detail, unlike the Examiner seems to imply, GM-CSF produced by one process is not identical with GM-CSF produced by another process. Glycoproteins differ in their glycosylation structure depending on which cell line is used (here the human cell line HEK 293) as well as the concentration of precursors in the culture conditions, as shown in the examples of the present invention, compare detailed explanation above.

The glycoprotein producible by a process with a specific selection process as claimed (human cell line NM-F9 with 90 mM ManNAc) to provide a specific sialic acid content in the novel highly active product structurally differs from **Sigma**'s GM-CSF.

Claim Rejections – 35 USC § 103 – Betenbaugh & ordinary skill as evidenced by Bonig, Claims 9, 24-30 and 33

Examiner asserts that the instant claims are "broadly drawn to a process for production of a highly active glycoprotein".

Applicant would like to stress the structural differences in the carbohydrate part of the highly active glycoprotein that the process results in, discussed under the response to the 102 rejections above.

Significantly, by employing the claimed process which **controls the degree of sialylation**, in contrast to **Betenbaugh**'s process , a glycoprotein with a human glycosylation pattern and an optimal degree of sialylation of high activity that structurally differs from prior art proteins is obtained.

Examiner asserts that **Betenbaugh**'s cells have a defect in adding sialic acid to a glycoprotein. Applicant respectfully disagrees.

More precisely, because **insect** cells **naturally** do not have a significant sialylation activity, **Betenbaugh** genetically engineers insect cells to **enable** the attachment of sialic acids to glycoproteins, providing such insect cells (and other cells which **lack** sialylation activity) with the ability to produce sialylated glycoproteins (in contrast to their natural ability).

Betenbaugh et al. discloses processes to make sialylated glycoproteins, and manipulating levels of CMP-SA by adding key precursors such as ManNAc. However, **Betenbaugh** does not disclose how the activity of a glycoprotein may be optimized by comparing the formed sialylated glycoprotein to a reference protein and selecting the glycoprotein with the optimal degree of sialylation and corresponding highest activity.

Notably, unlike the present invention, **Betenbaugh et al.** does not disclose that the optimal degree of glycoprotein sialylation for maximum glycoprotein activity may be below maximum sialylation. In contrast, **Betenbaugh et al.** adds precursors of CMP-SA to enhance the donor substrate CMP-A as high as possible to achieve a degree of

sialylation as high as possible.

Unlike the present claims, **Betenbaugh** is not concerned with obtaining glycoproteins that have a carbohydrate structure which has a sialylation degree that provides higher activity, but to enable the production of glycoproteins comprising sialic acids of any degree in cells which normally do not have sialylation activity.

In contrast, the present inventors introduced a defect into the sialic acid biosynthesis pathway of the expression cells that can be easily "rescued", thereby allowing to control sialylation activity via addition of the sialic acid precursor and determine and select the concentration that provides the highest activity.

Betenbaugh "cures" the natural condition of insect cells that naturally cannot produce sialylated glycoproteins while the present claims use human cells that are selected to have the very defect that Betenbaugh cures.

Starting with the present cells, **Betenbaugh**'s objective would lead nowhere, no matter which prior art document was combined with Betenbaugh, because his objective is already achieved – the human cells already produced sialylated proteins, before introduction of the defect to achieve the different objective of highly active human glycoproteins under the present claims.

Thus, the objectives of the present invention and Betenbaugh are diametrically opposed, and Betenbaugh is irrelevant – at best, Betenbaugh is teaching away from the present claims.

As Examiner correctly states, Betenbaugh teaches manipulating carbohydrate pathways “so that the cells produce complex sialyated glycoproteins useful for enhancing the value of eularyotic expression systems”, in case of eukaryote cells that happen to have the same defect as insect cells.

Again, the cells employed in the present claims in their wildtype form already formed complex sialylated glycoproteins (NM-F9 cells have been generated by glycoengineering of NM wt cells resulting in their **inability** to sialylate) – **Betenbaugh** would stop right there and use the wildtype cells, mission accomplished.

Arguendo, if one insisted on starting with such cells and happened to select a cell having a relevant metabolic defect, Betenbaugh does not disclose that a higher precursor concentration in the medium directly results in a higher sialylation degree.

Furthermore, once the optimal precursor concentration was determined, a comparative assay is necessary to determine the glycoprotein product with the highest activity. However, **Betenbaugh** also does not disclose comparative assays that would allow this.

The Examiner acknowledges that “Betenbaugh does not teach performing [an] assay using a sialylated protein and then optimiz[ing] how much to sialylate a protein to achieve higher or maximum activity”. The Examiner then uses **Bonig** to support the skill of the art in routinely achieving more activity by higher **glycosylation**.

Significantly, **Bonig** is directed **glycosylation** which is different from **sialylation**.

Glycosylation refers to the whole carbohydrate structure which may or may not contain sialic acids/be "sialylated" (usually at the ends of a tree-like structure of several carbohydrate units).

Notably, starting with the wildtype variant of the cells employed in the present process claims, before introduction of the defect in their biosynthesis, no such increased sialyation would be necessary if the cells were simply left in their natural wildtype state.

Accordingly, the routine skill of the art as demonstrated by Boning does not add anything to Betenbaugh, and does not arrive at the present invention.

Arguendo, if one insisted to using the defective cell rather then the wildtype (where Betenbaugh's objective was already achieved), one still would not arrive at the invention, because **Bonig** is directed to increasing overall **glycosylation**, not the formation of carbohydrate structures having a specific degree of sialic acid within their carbohydrate structures which provides glycoproteins with a high activity.

Furthermore, for glycoproteins carrying sialic acid residues, the prejudice in the art was that the higher the sialic acid content in the carbohydrate structure of a given glycoprotein, the higher is the glycoprotein's activity.

In contrast, the inventors in the present application disclose that an activity of a glycoprotein is highest at a specific degree of sialylation which is high but **below** the maximum degree of sialylation.

Accordingly, the claimed methods allow to control the degree of sialylation in product formation and to research, identify and produce the glycosylated product with the carbohydrate structure having a degree of sialylation which results in the highest activity of the product.

Structural variants of glycoproteins that differ in their degree of sialylation are compared to each other or to a reference in a bioassay in their desired activity/efficacy. Thereby, a correlation between the concentration of the sialic acid precursor additive in the culture medium, the degree of sialylation of the glycoprotein and the activity of the glycoprotein is determined that allows to choose the structural variant with the highest activity.

Selection of the precursor concentration resulting in - and thus, the sialylation variant having - the highest activity/efficacy allowed to form the inventive highly active structural glycoprotein variant of claim 35. By the claimed processes, glycoproteins of novel structure having an optimal degree of sialylation and therefore the desired activity/efficacy are obtained.

Unlike **Betenbaugh** or **Boning**, the claimed processes control the **degree of sialylation** by employing specific defective cells (rather than the wildtype which is fully functional in sialylation) and carefully choosing the concentration of the sialic acid precursor in the culture medium, detecting the activity of the glycoprotein, and comparing it to activities achieved at higher and lower precursor concentrations. A

higher precursor concentration in the culture medium directly results in a higher sialylation degree of the produced glycoproteins.

The relationship between precursor and sialylation concentration employed in the present claims is not taught or even hinted at by **Betenbaugh** .

The comparative assay of the present claims wherein the resulting glycoproteins that structurally differ in their degree of sialylation are compared to each other in their activity to achieve and demonstrate, for the first time, a structural variant not maximally sialylated with a higher activity.

As disclosed in the specification of the present application, the highest activity/efficacy of a glycoprotein is not synonymous with the maximum level of sialylation. Instead, high but below maximal **sialylation** (**not** glycosylation, unlike **Bonig**) can achieve the highest activity, compare example 6 as described in detail above.

The Examiner appears to misrepresent the teaching of **Bonig** in this regard. **Bonig** only describes that the protein G-CSF has a higher activity in **glycosylated** form than in **non-glycosylated** form. It is generally acknowledged in the art that proteins which are glycosylated in their natural form (such as G-CSF) loose at least some of their activity if they are not glycosylated.

However, the activity of glycosylated over non-glycosylated is irrelevant in the context of the present claims, since **all** formed proteins are g**lycosylated**; only their degree of **sialylation** differs.

In the claimed processes, glycosylated forms **differing in their degree of sialylation** of a glycoprotein of interest are compared to each other. The difference between these forms is not whether they are glycosylated or not (they are in fact all glycosylated), but rather the degree of sialic acid residues present in the glycosylation of said glycoprotein varies between the different forms of said glycoprotein.

Sialic acid is only one of several different monosaccharide units present in the glycosylation of proteins. In particular, natural glycosylation of proteins encompasses - besides sialic acid residues - for example galactose residues, mannose residues, glucosamine residues, and fucose residues. Thus, in the claimed method the amount of one specific component of the glycosylation is optimized to obtain glycoproteins with high activity, while Bonig et al. only refers to the presence or absence of the entire glycosylation.

The examiner asserts that it was prima facie obvious to produce glycoproteins in insect and mammalian cells with a defect and to co-express sialyltransferases to form sialylated glycoproteins.

The applicant respectfully disagrees for the reasons discussed above. The method as claimed in not prima facie obvious, as the cells without the defect already have what Betenbaugh tries to achieve. Even if one insisted to combine Bonig with Betenbaugh, which we note the Examienr refrains to do, the combination would not arrive at the invention for the reasons detailed above – Boning is not concerned with the

degree of **sialyation** but the degree of **glycosylation**; the two differ.

The examiner asserts that one of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success because **Betenbaugh** teaches making a number of proteins such as human transferrin in insect cells as well as human CHO cells.

Applicant respectfully disagrees. "CHO" in CHO cells stands for "Chinese Hamster Ovary", these cells are not human. **Betenbaugh** does not teach any human cells. More significantly, Betenbaugh does not teach the claimed method how to achieve highly active glycoproteins that differ in their sialylated glycostructure, and how to produce and select these particular glycoproteins.

As discussed above, the wildtype of the cells employed in the present invention, without the defect employed in the present method claims, will naturally produce the sialyated glycoproteins which are the objective of Betenbaugh et al.

Nowhere did **Betenbaugh**, **Bonig**, or any other prior art, alone or in combination, disclose or hint at the possibility to control the degree of sialylation via the concentration of the sialic acid precursor in the culture medium. The present claims further require using a cell line with a defect allowing for such control of the sialyation degree, and a comparative bioassay to select the sialylation form of the glycoprotein of interest having the highest activity/efficacy, which surprisingly is not the maximally sialylated one.

Accordingly, applicant believes the claims are inventive. Reconsideration is respectfully requested.

__Claim Rejections – 35 USC § 103a – Betenbaugh & Muramatsu & Fogolin, Claims 31, 32 and 34__

The Examiner acknowledges that **Betenbaugh** does not teach using NM-F9 cells to express GM-CSF.

The Examiner asserts that **Muramatsu** teaches F9 cells and further asserts that F9 "is another name [for] NM-F9".

Applicant respectfully disagrees. The F9 cells described by **Muramatsu et al.** are not identical to the NM-F9 cells used in the methods and products of the present claims and in US 7,595,192. **Muramatsu** refers to F9 embryonal carcinoma cells (see title and first paragraph of **Muramatsu**), a murine embryonal carcinoma cell line having the ATCC accession number CRL-1720. In contrast, the present application, as well as US 7,595,192 cited by the Examiner (notably authored by the inventors of the present application) refers to the human myelogenous leukemia cell line NM-F9 which is derived from the K562 cell line and deposited by the applicant according to the Budapest treaty under accession number DSM ACC2606 (see, e.g., US 7,595,192, column 14, line 46 et seq.).

Fogolin does not appear to add more than the prior art documents discussed

above, since, as the Examiner points out, it discloses that glycoprotein GM-CSF can be produced in various cells such as hamster and monkey cells (CHO, COS).

The Examiner's assertion as to the prima facie use of F9 "aka" NM-F9 cells as taught by **Muramatsu** to express GM-CSF taught by **Fogolin** is incorrect, as the cells are not the same despite their similar name.

Neither **Muramatsu** nor **Fogolin** add more to **Betenbaugh**, alone or in combination. As discussed for **Betenbaugh** in great detail above, none of the prior art documents discloses or hints at the possibility to control the amount of **sialylation** (which differs from **glycosylation**) via the concentration of the sialic acid precursor in the culture medium. The present claims further require using a cell line with a defect that allows for such control of the sialyation degree, and a comparative bioassay to select the sialylation form of the glycoprotein of interest having the highest activity/efficacy, which surprisingly is not the maximally sialylated one.

Accordingly, applicant believes the present claims are novel and unobvious.

Wherefore, early and favorable action is earnestly solicited.

EXTENSION OF TIME

Applicant hereby requests a two-month extension of time, the fee for which may be charged to Deposit Account no. 14-1263.

ADDITIONAL FEES

Please charge any insufficiency of fees, or credit any excess to Deposit Account No. 14-1263.

Respectfully submitted,

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